CHAPTER 4: SYNTHESIS OF CGE-1

4.1 Introduction

The designing of MIH antagonists establish an innovative class of growth enhancers for the stimulation of growth in crustaceans by significantly shortening of intermolt period. The CGE-1 was designed and synthesized to mimic the dipeptide at C-terminal segment of MIH and to block its binding action on MIH receptor. The importance of biological properties of molt inhibiting hormone and the role of residues critical for conferring the molt inhibiting activity has led to the discovery of several molecules. The virtual screening and manual alterations to create drug like molecules suggested that nitrogen-containing compounds, more specifically the amide compounds has shown a better in-silico activity as small molecule inhibitors of molt inhibiting hormone. The CGE-1 showed the best fit value and an excellent pharmacokinetic and pharmacodynamics activity was further considered for synthesis and validation. In this chapter, the designing of route map and syntheses of CGE-1 is presented. In spite of evidence of aquaculture utility by computer-assisted drug discovery, the mechanism of the therapeutic effect of CGE-1 can only be verified by in-vivo experiments. The synthesis of CGE-1 offers the possibility to reveal the various physiological roles of MIH in decapod crustaceans and to design a route map with better financial profiles that will lead to synthesize the compound in a cost effective method. As a part of this trend we
attempted the synthesis of novel amide containing compound as growth enhancing agents.

The biochemical properties of nitrogen containing compounds have gained much attention in biology and chemistry [1-8]. The activity of amides is well acknowledged in several biological pathways, drugs, and agrochemical molecules, and has urged comprehensive studies in the formation of the amide bond. Among those synthesis schemes, the use of acyl chlorides and N, N-Dicyclohexylcarbodiimide (DCC) along with the catalytic amount of DMAP (4-dimethylaminopyridine) is one of the easiest, cost effective yet robust methods for amide coupling in our research project [9]. Besides used for peptide synthesis, DCC have been used comprehensively in amide synthesis tested for various biological activities. In this chapter, we report a simple, accessible, widely used and an efficient method of CGE-1 synthesis using acid chlorides and DCC/DMAP as two different schemes.

4.2 Materials and Methods

The designed compound CGE-1 described in this study was prepared using two different route maps [Figure 4.1]. CGE-1 was synthesized following the method described in the literature for the synthesis of amides with minor modifications.

In the first method, the conventional DCC/DMAP method was used to synthesize the CGE-1 [10-14]. The dicyclohexyl carbodiimide (DCC) was used as a coupling agent for the ester synthesis. 3-Bromobenzoic acid (0.08M) and ethylenediamine (0.04M) was
dissolved in 250 mL of ethyl acetate, and then Dicyclohexylcarbodiimide (0.04M) was added along with a catalytic amount of dimethylaminopyridine (0.04M). The flask was fitted with calcium carbonate guard tube and the mixture was stirred for 3h at room temperature. Thin layer chromatography was performed after every 30 minutes to monitor the reaction progress and to observe the formation of the products by comparing the bands with the reactants used as reference. The dicyclohexyl urea formed as solid byproduct during the reaction was removed by filtration. The filtrate containing the CGE-1 was washed with 10% HCl, 10% potassium carbonate and with brine. After washing the filtrate was dried over anhydrous sodium sulfate and applied to column chromatography and eluted with various proportions of chloroform and petroleum ether. The purity and structure of CGE-1 was confirmed using \(^1\)H and \(^{13}\)C-NMR.

**Figure 4.1: Route map for the synthesis of growth enhancer (CGE-1)**

**Scheme-1**

3-Bromobenzoic acid \(\xrightarrow{\text{PCl}_3}\) Bromobenzoyl Chloride + Ethylene diamine

**Scheme-2**

3-Bromobenzoic acid + Ethylene diamine \(\xrightarrow{\text{DCC/DMAP, 3hr. in RT}}\) Ethyl acetate

\(\xrightarrow{\text{Ice Bath, Dry Acetone, Pyridine}}\) N,N-diethane-1,2-diylbis(3-bromobenzamide)

**DCC:** N, N-Dicyclohexylcarbodiimide  
**DMAP:** 4-Dimethylaminopyridine
In the scheme-2, The CGE-1 was also synthesized by acid chloride method. Initially, 3-Bromobenzoic acid was treated with PCls which yielded 3-bromobenzoyl chloride. Two equivalent of 3-bromobenzoyl chloride was then added drop wise to the one equivalent of ethylamine in dry acetone in the presence of pyridine as potential acid scavengers. Since, this reaction is very violent, hence it was performed in ice bath under constant stirring condition. After the completion of reaction, the mixture was filtered to remove pyridinium chloride formed during the synthesis. The compound was subsequently purified by column chromatography and structure was confirmed with $^1$H and $^{13}$C-NMR.

4.3 Results and Discussion

The crucial stage of the amide synthesis is a coupling reaction between ethylamine and 3-bromobenzoic acid using DCC/DMAP as a coupling agent. The first step of reaction includes the reaction of the 3-bromobenzoic acid with DCC to form the O-acylurea. This intermediate then yield the amide through direct coupling with the ethylene diamine and a by-product Dicyclohexylurea (DCU) formed which was insoluble in the reaction solvent and was removed through filtration. A higher yield was obtained when the reaction mixture was kept at 0°C for five minutes as it possibly enhances the esterification and it is also well known that the addition of DMAP is crucial for the formation of esters.
The reaction of acid chloride with ethylamine ensues in two stages. In the first step, the Ethylamine reacts with the 3-bromobenzoyl chloride to give respective amide and hydrogen chloride gas. In the next step, the hydrogen chloride gas produced during the reaction than reacts with excess pyridine to give pyridinium chloride. Anhydrous condition was maintained as it did not allow the HCl to get into the reaction mixture by dissolving in water and also, chilled reaction condition was maintained using ice-bath which typically enhances the yield.

The structure of CGE-1 was confirmed by the appearance of protons and carbons in their distinctive position in NMR. The presence of aliphatic and aromatic protons was confirmed using their integration values in $^1$H-NMR [Figure 4.2].

![Figure 4.2: Characterization of CGE-1 using $^1$HNMR Spectra. The distribution of characteristic aromatic and aliphatic protons according to their occurrence in those regions was confirmed with proton NMR.](image-url)
As expected, a single sharp peak on aliphatic region between 3-4 ppm along with doublet and multiplets at aromatic region between 7-9 ppm. Similarly, the pattern of appearance of carbon moieties at $^{13}$C-NMR was confirms the structure of CGE-1 [Figure 4.3]. The aliphatic carbon was appeared between 30-40 ppm while the aromatic carbon was observed between 120-140 ppm. The formation of C=O was also confirmed which was characteristically appeared at 167 ppm.

Figure 4.3: $^{13}$C NMR Spectra of CGE-1. The carbon NMR spectrum was used to confirm the two benzene rings along with the diamine aliphatic region according to their prediction space in the spectra.
4.4 Conclusion

In conclusion, we have established economically sustainable methods for the synthesis of novel growth enhancer, CGE-1. The synthesis of CGE-1 in the case of DCC/DMAP method was high yielding as compared to the acid chloride method probably due to the possibility of other side reaction as well as racemization, hydrolysis and deprotection during the reaction. We optimized the condition for acid chloride method using various reports which suggested that use of aqueous inorganic bases prompt hydrolysis and organic bases cause ketene formation if an α-proton is present. Hence, formation of amide bond in anhydrous condition with a hydrogen chloride scavenger can eliminate these limitations in the use of acyl chlorides. This is the first report for the synthesis of CGE-1 which is a simple and highly effective using pyridine as acid scavenger under anhydrous condition with noteworthy enhancement over acknowledged procedures.

4.5 References


